Methadone - not a magic bullet in melanoma therapy

Brüggen, Marie-Charlotte ; Mangana, Joanna ; Irmisch, Anja ; French, Lars E ; Levesque, Mitchell P ; Cheng, Phil F ; Dummer, Reinhard

Abstract: Methadone (Met) mainly acts as a -opioid receptor agonist. Recent evidence pointing towards the role of Met in sensitization of certain cancer cell lines to chemotherapeutic agents has promoted the hypothesis that Met may be a useful adjuvant to cancer chemotherapy. We wanted to address whether Met has, alone or in combination with a chemotherapeutic agent, an effect on melanoma cell viability in vitro. Only a small fraction (4.3%) of our 102 melanoma biobank cell lines with RNA sequencing data showed expression of the main receptor for Met (OPRM1). We assessed the viability of melanoma cell lines with high, medium or low/no OPRM1 expression (OPRM1 , OPRM1 , OPRM1) 72 hours after treatment with Met alone or combined with cisplatin (Cis). Our analyses show that Met alone did not affect cell viability. While Cis/Met treatment did not have an effect on viability of OPRM1 or OPRM1 cell lines, it resulted in a slightly decreased cell viability of OPRM1 cells. Clinically, concurrent temozolomide/Met treatment did not have an effect in our single-case report of a patient suffering from uveal melanoma. Taken together, our findings do not provide evidence for recommending Met as an adjuvant to chemotherapy in melanoma patients.

DOI: https://doi.org/10.1111/exd.13543

DR. MARIE-CHARLOTTE BRÜGGEN (Orcid ID : 0000-0002-8607-6254)

Article type : Methods Letter to the Editors

Title:

Methadone - not a magic bullet in melanoma therapy

Authors:

Marie-Charlotte Brüggen[1, 2], Joanna Mangana[1, 2], Anja Irmisch[1, 2], Lars E. French[1, 2], Mitchell P. Levesque[1, 2], Phil F Cheng[1, 2*], Reinhard Dummer[1, 2*]

Affiliations:

[1] Department of Dermatology, Zurich University Hospital, Zurich, Switzerland

[2] Faculty of Medicine, University of Zurich, Zurich, Switzerland

* shared last authorship

Corresponding author's address

Reinhard Dummer

Department of Dermatology

University Hospital Zurich

Gloriastrasse 31

CH – 8091 Zurich

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/exd.13543

Tel.: +41 (0)44 255 93 81

Email: reinhard.dummer@usz.ch;

ABSTRACT

Methadone (Met) mainly acts as a μ-opioid receptor agonist. Recent evidence pointing towards the role of Met in sensitization of certain cancer cell lines to chemotherapeutic agents has promoted the hypothesis that Met may be a useful adjuvant to cancer chemotherapy. We wanted to address whether Met has, alone or in combination with a chemotherapeutic agent, an effect on melanoma cell viability *in vitro*. Only a small fraction (4.3%) of our 102 melanoma biobank cell lines with RNA sequencing data showed expression of the main receptor for Met (OPRM1). We assessed the viability of melanoma cell lines with high, medium or low/no OPRM1 expression ($OPRM1^{high}$, $OPRM1^{med}$, $OPRM1^{neg}$) 72 hours after treatment with Met alone or combined with cisplatin (Cis). Our analyses show that Met alone did not affect cell viability. While Cis/Met treatment did not have an effect on viability of $OPRM1^{med}$ or $OPRM1^{neg}$ cell lines, it resulted in a slightly decreased cell viability of $OPRM1^{high}$ cells. Clinically, concurrent temozolomide/Met treatment did not have an effect in our single-case report of a patient suffering from uveal melanoma. Taken together, our findings do not provide evidence for recommending Met as an adjuvant to chemotherapy in melanoma patients.

BACKGROUND

Methadone (D,L Methadone; Met) is a long-acting μ-opioid receptor (OPRM1) agonist (*1*). Met is mainly known and used as a heroine substitute, additional administration has been reported in the management of cancer pain (*1*).

A series of studies (*2-9*) has pointed towards an antitumoral effect of Met in various types of human cancer. *In vitro* data suggest that Met alone can induce apoptosis (*5, 6*), and, when combined with chemotherapeutic agents, sensitize cancer cells for their actions, possibly in an OPRM1-dependent fashion (*8*). Clinically, a retrospective, non placebo-controlled study has pointed towards a sensitizing role of Met for temozolomide (TMZ) chemotherapy in glioblastoma (*9*).

These studies have recently received significant attention, resulting in numerous patient requests for Met. In melanoma, the potential for Met to sensitize patients for chemotherapy has not yet been addressed.

QUESTIONS ADDRESSED

We thus sought to investigate how the concept of Met as a sensitizer for chemotherapy translates to melanoma and may justify its clinical use. Our main aim was to evaluate whether in an *in vitro* setting, Met has, either alone or in combination with a chemotherapeutic agent, an effect on melanoma cell viability. The related questions were whether Met could 1) directly have an effect on the viability of melanoma cell lines and/or 2) indirectly sensitize melanoma cells to chemotherapeutic agents such as Cis.

EXPERIMENTAL DESIGN

Details on the subsequently described methods, cell lines and reagents are given in *Supplementary Material and Methods*.

Cell lines, Chemicals

Patients gave their written informed consent approved by the local institutional review board (EK647/800). Primary melanoma cell cultures were established from metastatic melanoma tissue as previously described (*10*).

Cell viability assay

The cell lines were challenged with dose-escalating concentrations of Met alone, Cis alone, Cis/Met combined, an ERK-inhibitor or ERK-inhibitor/Met combined for 72 hours. Cell viability was estimated using a fluorometric (resazurin) assay.

Immunohistochemistry stainings

Immunohistochemical stainings were performed as previously described (*11*). We used an anti-OPRM1 antibody (PA5-26138; Thermo Fisher Scientific, Reinach, Switzerland), negative controls were obtained by substituting rabbit IgG for the primary antibody.

RESULTS

OPRM1 is lowly expressed in most melanoma cell lines

Met binds to OPRM1, acting as an agonist . We first assess OPRM1 expression in melanoma based on RNAseq data from 102 cell lines of our URPP primary melanoma cell line biobank. Only four cell lines (4.3%) showed high ($OPRM1^{high}$) and 58 (63%) medium OPRM1 ($OPRM1^{med}$) expression, the other cell lines were OPRM1-negative ($OPRM1^{neg}$). We chose an $OPRM1^{high}$, $OPRM1^{med}$ and $OPRM1^{neg}$ melanoma cell line to test the effects of

Met (Figure 1A). Immunohistochemistry stainings confirmed the expression of OPRM1 at the protein level (Figure 1B).

Met treatment does not significantly affect melanoma cell viability

To investigate the effects of Met on these melanoma cell lines with regard to viability, we treated them with Met, Cis (positive control), medium (negative control) or a combination of Cis/Met using dose-escalating concentrations. A cell viability assay was performed after 72 hours. In a series of three independent experiments, our results consistently showed that in $OPRM1^{med}$ and $OPRM1^{neg}$ melanoma cell lines, Met alone or in combination with Cis did not have any effect on cell viability (Figure 1C, D). In the $OPRM1^{high}$ melanoma cell line, we observed a slight decrease in cell viability (ranging from 10-20%) with Cis/Met as compared to Cis alone, but median dose effect (IC_{50}) values were (when ANOVA one-way was applicable) not significantly different (Figure 1C). When using an ERK-inhibitor instead of Cis, we did not observe any synergistic effect of Met on cell viability (data not shown).

Single-case study: metastatic uveal melanoma

A 60 year-old patient with stage IV metastatic GNA11 mutant uveal melanoma (pTxN0M1c) showed progressive disease, i.e. new bone and extensive liver metastases following 4 cycles of combined immunotherapy (ipilimumab 200mg, nivolumab 80mg). Previously, he had been treated with a pan-Raf-inhibitor and selective internal radiotherapy for his liver metastases. He was put on TMZ (200mg/m^2 body surface area; 380mg daily per os, 5 days/cycle). Concurrently to TMZ, Met was perscribed upon his request (10mg/ml, 30-0-30-0 drops daily per os).

Despite three cycles of TMZ/Met, he deteriorated clinically and was admitted to the hospital experiencing weight loss, asthenia, abdominal distension and peripheral edema. Lactate dehydrogenase levels had doubled (5017 U/l) and S-100 protein was elevated (0.45 ug/l). Computer tomography confirmed rapid disease progression in the liver with ascites and peritoneal carcinomatosis. The patient declined further melanoma-specific treatment and best supportive care was iniated. He died from liver failure two weeks upon admission.

CONCLUSIONS

Based on a series of studies (*2-9*) suggesting a role of Met as a chemotherapy sensitizer in certain cancer types, we addressed the potential/validity of this hypothesis in the setting of melanoma.

Our results show high OPRM1 expression in only a minority of primary melanoma cultures obtained from patients with metastatic melanoma. Although we observed a slight decrease in cell viability (10-20%) in OPRM1^{high} melanoma cells 72 hours after Cis/Met application (vs. Cis alone). Moreover, Cis/Met did not have any effect on OPRM1^{neg} and OPRM1^{med} melanoma cell lines. These *in vitro* findings indicate that 1) OPRM1 is expressed in a small subset of metastatic melanomas only and that 2) regardless of OPRM1 expression (neg/med/high), Met alone or in combination does not have a significant effect on melanoma cell viability.

Whereas in various cancer types, OPRM1 expression has been associated with higher staging and/or poor outcome (*8, 12-15*), our findings suggest this is not the case in melanoma. As to the effects of Met, Friesen et al. (*2-4*) had reported that apoptosis rates increased by about 50% in leukemia and glioblastoma cell lines treated with Met combined

with chemotherapeutic agents. Importantly, Met concentration ranges (including therapeutic range) used by Friesen et al were covered in our experiments. The divergent results could, at least partially, be explained by the use of different cancer cell lines, but clearly show that Met does not have the potential to significantly impact cell viability in melanoma. Clinically, our single-case experience (concurrent treatment with TMZ/Met) showed a lack of effect in uveal melanoma.

In conclusion, it seems unlikely that combining Met with chemotherapy has a beneficial effect for melanoma patients. Our findings do not provide evidence for recommending the use of Met as an adjuvant to chemotherapy to our melanoma patients.

ACKNOWLEDGEMENTS

We thank Katharina Eisoldt, Mirka Schmid and Alice Meier for technical support.

AUTHOR CONTRIBUTION

M.-C.B. performed and designed research, analyzed the data and wrote the paper. J.M. and A.I. contributed essential tools, M.L., L.E.F., P.C. and R.D. designed the study and wrote the paper. No conflicts of interest to be reported.

REFERENCES

1. P. J. Wiffen, B. Wee, S. Derry, R. F. Bell, R. A. Moore, Opioids for cancer pain - an overview of Cochrane reviews. *Cochrane Database Syst Rev* **7**, CD012592 (Jul 06, 2017).

2. C. Friesen, I. Hormann, M. Roscher, I. Fichtner, A. Alt, R. Hilger, K. M. Debatin, E. Miltner, Opioid receptor activation triggering downregulation of cAMP improves effectiveness of anti-cancer drugs in treatment of glioblastoma. *Cell Cycle* **13**, 1560 (2014).

3. C. Friesen, M. Roscher, A. Alt, E. Miltner, Methadone, commonly used as maintenance medication for outpatient treatment of opioid dependence, kills leukemia cells and overcomes chemoresistance. *Cancer Res* **68**, 6059 (Aug 01, 2008).

4. C. Friesen, M. Roscher, I. Hormann, I. Fichtner, A. Alt, R. A. Hilger, K. M. Debatin, E. Miltner, Cell death sensitization of leukemia cells by opioid receptor activation. *Oncotarget* **4**, 677 (May, 2013).

5. R. Maneckjee, J. D. Minna, Nonconventional opioid binding sites mediate growth inhibitory effects of methadone on human lung cancer cells. *Proc Natl Acad Sci U S A* **89**, 1169 (Feb 15, 1992).

6. W. L. Heusch, R. Maneckjee, Effects of bombesin on methadone-induced apoptosis of human lung cancer cells. *Cancer Lett* **136**, 177 (Mar 1, 1999).

7. A. Singh, A. Jayanthan, A. Farran, A. N. Elwi, S. W. Kim, P. Farran, A. Narendran, Induction of apoptosis in pediatric acute lymphoblastic leukemia (ALL) cells by the therapeutic opioid methadone and effective synergy with Bcl-2 inhibition. *Leuk Res* **35**, 1649 (Dec, 2011).

8. S. M. Kang, J. L. Rosales, V. Meier-Stephenson, S. Kim, K. Y. Lee, A. Narendran, Genome-wide loss-of-function genetic screening identifies opioid receptor mu1 as a key regulator of L-asparaginase resistance in pediatric acute lymphoblastic leukemia. *Oncogene* **36**, 5910 (Oct 19, 2017).

9. J. Onken, C. Friesen, P. Vajkoczy, M. Misch, Safety and Tolerance of D,L-Methadone in Combination with Chemotherapy in Patients with Glioma. *Anticancer Res* **37**, 1227 (Mar, 2017).

10. M. I. Raaijmakers, D. S. Widmer, M. Maudrich, T. Koch, A. Langer, A. Flace, C. Schnyder, R. Dummer, M. P. Levesque, A new live-cell biobank workflow efficiently recovers heterogeneous melanoma cells from native biopsies. *Exp Dermatol* **24**, 377 (May, 2015).

11. M. C. Bruggen, I. Klein, H. Greinix, W. Bauer, Z. Kuzmina, W. Rabitsch, P. Kalhs, P. Petzelbauer, R. Knobler, G. Stingl, G. Stary, Diverse T-cell responses characterize the different manifestations of cutaneous graft-versus-host disease. *Blood* **123**, 290 (Jan 9, 2014).

12. Y. F. Zhang, Q. X. Xu, L. D. Liao, X. E. Xu, J. Y. Wu, J. Shen, Z. Y. Wu, J. H. Shen, E. M. Li, L. Y. Xu, kappa-Opioid receptor in the nucleus is a novel prognostic factor of esophageal squamous cell carcinoma. *Hum Pathol* **44**, 1756 (Sep, 2013).

13. D. Zylla, B. L. Gourley, D. Vang, S. Jackson, S. Boatman, B. Lindgren, M. A. Kuskowski, C. Le, K. Gupta, P. Gupta, Opioid requirement, opioid receptor expression, and clinical outcomes in patients with advanced prostate cancer. *Cancer* **119**, 4103 (Dec 1, 2013).

14. Y. S. Yao, R. Y. Yao, L. K. Zhuang, W. W. Qi, J. Lv, F. Zhou, W. S. Qiu, L. Yue, MOR1 expression in gastric cancer: a biomarker associated with poor outcome. *Clin Transl Sci* **8**, 137 (Apr, 2015).

15. C. Connolly, S. F. Madden, D. J. Buggy, H. C. Gallagher, Expression of anaesthetic and analgesic drug target genes in excised breast tumour tissue: Association with clinical disease recurrence or metastasis. *PLoS One* **12**, e0177105 (2017).

FIGURE LEGENDS

Figure 1.

(A) Expression of OPRM1 (on the y axis, in log(counts per million) assessed by RNA sequencing in 102 melanoma cell lines of our URPP biobank. Each dot corresponds to an individual melanoma cell line. The cell lines we chose for our experiments are highlighted (red frame), i.e. $OPRM1^{neg}$ (MM000921), $OPRM1^{med}$ (MM121008) and $OPRM1^{high}$ (MM141095). (B) Pictures of immunohistochemical OPRM1 staining of in the melanoma cell lines ($OPRM1^{neg}$, $OPRM1^{med}$ and $OPRM1^{high}$) we chose for our experiments. An isotype control and positive control (tonsil) are depicted in the left panel. (C) Changes in cell viability measured by a resazurin assay (results pooled from 3 independent experiments). Dose–response curves for the melanoma cell lines ($OPRM1^{neg}$, $OPRM1^{med}$ and $OPRM1^{high}$) treated for 72 hours with Met, Cis or a combination of Met/Cis in a dose-escalating manner. Values are defined as means converted to percentages after normalization to untreated control; error bars represent SEM. (C) Median dose effect in nM (analogue of IC_{50}) depicted for each melanoma cell line.

Figure 1.